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USE OF ANTI-IDIOTYPES AND SYNTHETIC PEPTIDES FOR CONTROL OF HUMAN T-LYMPHOTROPIC VIRUS TYPE III INFECTIONS

FINAL PROGRESS REPORT

by

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During the past two years, we have investigated the potential use of synthetic peptides and anti-idiotypes (anti-Id) for controlling HIV infection. We have identified four regions of the human immunodeficiency virus type 1 HIV-1 envelope glycoprotein that have the capacity to induce neutralizing antibody responses in experimental animals. Synthetic peptides corresponding to amino acid sequences to envelope glycoproteins gp120 and gp41 were used to identify these neutralizing epitopes. One peptide corresponding to amino acid sequences 735 to 752 from gp160 was coupled to a carrier protein and used to immunize chimpanzees. The chimpanzees produced an anti-gp160 response when immunized with this peptide; however, the neutralizing antibody response was weak. These chimpanzees, along with a control peptide immunized animal, were challenged intravenously with infectious HIV. Following challenge, the chimpanzees seroconverted and developed antibody responses against gag protein products. These studies indicated that chimpanzees were not protected from HIV infection by immunization with a

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single synthetic peptide that previously induced neutralizing antibodies in small experimental animals. Mouse monoclonal antibodies were generated to peptide 735-752. These antibodies inhibited cell fusion of divergent HIV-1 isolates but failed to inhibit HIV-2. No neutralization by these monoclonal antibodies was observed in a VSV (HIV-1) pseudotype assay.

We also have identified two gp41 synthetic peptides that exert a profound suppression of normal human proliferative responses to mitogens and allo-antigens along with immunosuppression of natural killer (NK) cell activity. Similar suppressive effects have been previously reported with a synthetic peptide analogous to amino acid sequences from the feline leukemia virus transmembrane glycoprotein.

Studies have utilized an affinity purified chimpanzee anti-gp41 as an Ab-1 preparation to generate polyclonal and monoclonal anti-Id. This anti-Id was serologically characterized as representing noninternal image Ab-2 preparations. BALB/c mice immunized with the anti-Id produced an Ab-3 like response which bound HIV gp41. The anti-Id induced anti-gp41 expressed a silent idiotype which was not expressed when BALB/c mice were immunized with a recombinant gp160. Thus, this anti-Id preparation can alter the serological characteristics of the immune response to gp160 in mice.

Finally, we have generated a mouse monoclonal anti-Id against a mouse monoclonal anti-CD4. This anti-Id exhibited internal image characteristics and mimicked CD4, the cellular receptor for HIV. The monoclonal anti-Id bound HIV gp120 and possessed in vitro neutralizing activity. A polyclonal anti-Id response in BALB/c mice immunized with anti-Leu3a had the capacity to neutralize four divergent HIV-1 isolates along with an HIV-2 isolate. In addition, baboons immunized with a monoclonal anti-CD4 produced an anti-Id response that recognized HIV gp120. These data suggest the vaccine possibility of an anti-Id response to a monoclonal anti-CD4 preparation.

Keywords: Monoclonal antibodies, Vaccines, Acquired Immune deficiency Syndrome (AW)

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In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

A. Immunogenicity of HIV-1 env Synthetic Peptides

Antigenic determinants are both sequential and conformational in nature. The ability to synthesize peptides predicted to contain these sequential epitopes allowed us to evaluate those domains on gp120 and gp41 which would be important in neutralization of the virus. We have identified several such regions of the HIV-1 envelope that correspond to neutralizing antigenic determinants. Peptides to be synthesized were selected by a computer model system that integrates both predicted hydrophilic and conformational information. Peptide 735-752 represented the most hydrophilic domain within gp41. Antibodies from hyperimmunized rabbits recognized gp41 as shown by immunoblotting and the env precursor (gp160) by RIP/SDS-PAGE. This result indicated that antibodies were reactive to both denatured antigen and to the more native conformational state seen by RIP analysis. Antibodies from five patients tested recognized this peptide and the specificity could be confirmed by competitive binding assays. Later studies suggested that approximately 15-20% of seropositive HIV-1 infected individuals recognized this peptide.

A second peptide, 503-532, consisting of the carboxyl end of gp120 and included residues from the amino terminus of gp41, was synthesized and rabbit antibodies to this peptide detected gp120 by both immunoblotting and radioimmunoprecipitation techniques. Again, antibodies from HIV-1 infected humans could detect this peptide, indicating that this peptide represents an immunogenic epitope in natural infection. Peptide 735-752 and 503-532 both elicited antibodies that neutralized the HTLV-IIIB and NY-5 strain of HIV-1, suggesting that these domains may be useful in developing HIV-1 vaccines.

We have also identified two other regions within the HIV-1 envelope glycoprotein that represent neutralizing epitopes. Antisera generated against these synthetic peptides analogous to these HIV-1 envelope amino acid sequences corresponding to regions 304 to 327 and 616 to 632 also neutralized HIV-1 infectivity in vitro as assessed by reduction of reverse transcriptase activity and syncytium inhibition.

Monoclonal antibodies were generated against synthetic peptides analogus to amino acid sequences 735-752. Three IgM monoclonal antibodies were found to neutralize different HIV-1 isolates but not HIV-2 isolates, as determined by syncytial inhibition assays. VSV (HIV-1) pseudotypes, however, were not neutralized, indicating that gp41 was not accessible to these antibodies on the pseudotype particles. The antibodies appeared to affect early steps in adsorption and penetration of HIV-1.

B. HIV-1 Synthetic Peptide Vaccine Studies in Chimpanzees

Peptide 735-752 analogous to gp160 amino acid sequences of HIV-1 was used to immunize two chimpanzees to evaluate if the humoral response to HIV-1 would protect this species from infection. Four immunizations of the peptide coupled to a carrier protein were performed followed by intravenous inoculation of purified cell-free virus. Prior to inoculation, antibodies from these animals were shown to react to gp41 by immunoblotting and gp160 by RIP/SDS-PAGE, indicating that humoral responses in chimpanzees were elicited. A third chimpanzee immunized with a control peptide was also challenged with virus. The humoral response was monitored on a weekly basis for the first 12 weeks following HIV infectious challenge and post-inoculation reactivity to viral

proteins was observed for all three primates. Antibodies to gp120, p55, and p24 were observed in all chimpanzees challenged with HIV. These data indicate that a single HIV-1 envelope glycoprotein synthetic peptide did not induce protective immunity against HIV-1 infection in chimpanzees.

C. HIV-induced Immunosuppression

We have recently demonstrated that two synthetic peptides homologous to HIV gp160 amino acid sequences 735-752 and 840-860, respectively, exert a pronounced suppression of mitogen-induced blastogenic response in vitro. The mechanism of immunosuppression remains unclear; however, our data suggest that suppression occurs at the level of IL-2 T cell interaction and that a down regulation of both IL-2 production and responsiveness may occur in HIV-peptide treated normal peripheral blood mononuclear cells. In this study, peptides conjugated to protein carriers, but not free peptides, exerted a profound suppression of the normal human lymphocyte proliferative response to ConA, PHA, PWM and alloantigens. A synthetic peptide corresponding to a 17 amino acid sequence of the HIV TAT_{III} gene product had no suppressive effects. These results suggest that, in addition to the selective cytopathic effects of HIV on CD4 bearing T-cells, viral peptide-mediated immunosuppression may also play an important role in the pathogenesis of the disease.

We also examined the ability of peptides 735-752 and 846-860, respectively, to inhibit normal natural killer (NK) cell activity in vitro. Significant inhibition was observed by the two peptides on the normal NK cell activity as assessed against K562 tumor target cells in an in vitro radiolabeled Cr-release assay. This suppression of NK cell activity was observed only when the synthetic peptides were coupled to carrier proteins. No suppressive effects were observed when control peptides were similarly coupled to carrier proteins. Suppression of NK activity by both peptides was partially restored with the addition of exogenous recombinant human interleukin-2 (IL-2). The formation of effector cell-target cell conjugates were not affected by peptides 735-752 and 846-860 as determined by binding experiments. This suggests that one potential mechanism of the observed NK suppression is the inhibitory effect(s) subsequent to the formation of the lytic complex. These results suggest that the two peptides corresponding to sequences within the HIV transmembrane gp41 may play an important role in the pathogenesis of the defective NK cell activity observed in patients with AIDS.

D. Anti-idiotypes Induce an Anti-HIV Response

We have produced in rabbits anti-idiotypic antibodies (anti-Id) against chimpanzee antibodies directed against a synthetic peptide corresponding to a native epitope associated with gp41 of HIV-1. The peptide was analogous to amino acid sequence 735-752 from gp160. Characteristics of the anti-Id preparation included: (i) detection of a shared determinant present on a second chimpanzee and one out of three rabbit antibody preparations directed against the synthetic peptide; (ii) failure to recognize an idiotype in BALB/c mouse antisera to the peptide; and (iii) partial inhibition of the homologous chimpanzee idiotype (Id) preparation from binding either the peptide or a recombinant HIV-1 gp160 preparation. Immunization of BALB/c mice with the anti-Id induced an anti-peptide response which bound a recombinant gp160 preparation without subsequent peptide or gp160 exposure. The anti-gp160 containing sera from mice immunized with anti-Id were used to inhibit the Id-anti-Id reaction,

indicating that Id positive antibody response was induced. This Id is not normally expressed in the murine anti-gp160 immune response to the synthetic peptide and suggests that this anti-Id may activate normally silent clones. This study indicates that Id networks may be operational during the immune response to HIV-1 epitopes. In addition, non-internal image anti-Id preparations may be useful in altering the serological characteristics of an antibody response to HIV-1 relative to the nominal antigen.

We have also generated and characterized a monoclonal anti-idiotypic antibody (anti-Id) against a chimpanzee anti-gp41 Ab-1 preparation specific for synthetic peptide 735-752. This monoclonal anti-Id appeared to represent a non-internal image subclass of anti-Id. Characteristics of the monoclonal anti-Id preparation include: 1) ability to recognize the homologous chimpanzee anti-gp41 preparation along with a second heterologous chimpanzee anti-gp41; 2) failed to recognize an interspecies Id expressed on rabbit and mouse monoclonal anti-gp41 preparations similarly induced by immunizations with peptide 735-752; and 3) inability to inhibit the chimpanzee Ab-1 binding to peptide 735-752.

BALB/c mice and rabbits were immunized with this monoclonal anti-Id preparation. Mice produced an Ab-3 response that recognized peptide 735-752 and a recombinant gp160 preparation by ELISA. Sera containing the anti-gp160 from mice immunized with the anti-Id inhibited the Id-anti-Id reaction, indicating that Id positive antibody response was induced. The murine anti-Id induced anti-HIV response that also detected gp41 by Western blot. Immunization with peptide 735-752 induced a murine Ab-3 response expressing an Id that is not normally expressed during the murine anti-gp41 response. BALB/c mice immunized with this non-internal image monoclonal anti-Id produced an Ag positive, Id positive response. This Id however, is normally dormant during the immune response to the nominal antigen. Silent Id clones appeared to be activated within BALB/c mice when immunized with this anti-Id.

When rabbits were immunized with the monoclonal anti-Id preparation they produced an Ab-3 response that failed to bind either peptide 735-752 or HIV antigens.

Based on the above date, it was concluded that the monoclonal anti-Id did not exhibit any internal image activity and appeared to recognize a common non-antigen combining site Id present on two chimpanzee anti-gp41 preparations. The anti-Id failed to induce an anti-HIV response across species barriers. Failure of this anti-Id to induce an anti-HIV response across species barriers suggests its poor potential as a vaccine candidate. However, the rabbit Ab-3 expressed an Id that was similar to that expressed by the chimpanzee Ab-1. Rabbits immunized with peptide 735-752 failed to express and Id similar to that shared by the chimpanzee Ab-1 and rabbit Ab-3. Within a given host it appears that non-internal image anti-Id may have the capacity to preprogram the Id response. The selection of the particular Id to be expressed during the Ab-3 response is based on the Id of the Ab-1 recognized by the non-internal image anti-Id.

E. Idiotypes Present on Monoclonal Anti-CD4 Preparations

We have also generated a mouse monoclonal anti-Id against the mouse monoclonal anti-CD4 preparation, anti-Leu-3a. This anti-Id did not react with

a panel of irrelevant mouse monoclonal antibodies, indicating that neither anti-isotype nor anti-allotype specificities were recognized. The anti-Id inhibited the ability of anti-Leu-3a to stain $CD4^+$ T cells, suggesting that the anti-Id recognized an antibody combining site related determinant on anti-Leu-3a. This anti-Id bound HIV-1 determinants in the following assays: (i) viable membrane immunofluorescence of HIV-1 infected cells; (ii) commercial ELISA's; and (iii) Western blot analysis. In addition, the anti-Id partially neutralized HIV-1 infection of T cells in vitro. These results suggest that the anti-Id reacts with an Id determinant on anti-Leu-3a and mimics part(s) of the CD4 molecule that represents the viral receptor for HIV-1.

We have also produced a polyclonal anti-Id response to anti-Leu-3a in BALB/c mice. This polyclonal anti-Id exhibited in vitro neutralizing activity against four divergent HIV-1 isolates (HTLV-IIIB, ARV-2, MN, and RF) along with an HIV-2 isolate ($HIV\text{-}2_{ROD}$). The anti-Id recognized anti-Leu-3a, but failed to bind another anti-human CD4 preparation (OKT4) which does not inhibit HIV binding to the CD4 molecule. In addition, the anti-Id bound gp160 in a solid phase immunoassay. Together, these results have implications for a potential AIDS vaccine utilizing anti-CD4 preparations to induce an anti-Id response with the capacity to bind HIV at its receptor site.

Two baboons were also immunized with OKT4A, a murine monoclonal anti-CD4 that blocks the HIV gp120-CD4 interaction. Both baboons produced an anti-Id response that was specific for OKT4A. This anti-Id response also recognized other monoclonal anti-CD4, but failed to bind a series of irrelevant mouse monoclonal antibodies. The anti-Id inhibited the binding of OKT4A and anti-Leu-3a to $CD4^+$ cells, and stained HIV infected cells by viable membrane immunofluorescence. In addition, this anti-Id also bound HIV-1 envelope glycoproteins gp120 and gp160 by immunoblot analysis and recognized a recombinant gp160 preparation by a solid-phase immunoassay. Of particular interest, the anti-Id reacted with a band of 120 Kd in immunoblot employing simian immunodeficiency virus (SIV) antigens. Peripheral blood lymphocytes (PBL) obtained from the OKT4A-immune baboons proliferated in vitro to HIV antigens, suggesting the induction of a cell-mediated immune response. The surface phenotypes of the baboons' PBL and their in vitro proliferative response to mitogens were comparable to those of normal baboon PBL. These results suggest that anti-CD4 immunization did not result in T lymphocyte depletion or T lymphocyte anergy in these baboons. Further studies are required to assess the potential of anti-CD4 preparations as Id-based vaccines for controlling HIV infection.

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